Formic Acid Treatment for Control of *Varroa destructor* (Mesostigmata: Varroidae) and Safety to *Apis mellifera* (Hymenoptera: Apidae) Under Southern United States Conditions

PATTI J. ELZEN,[1] DAVID WESTERVELT,[2] AND RAYMOND LUCAS[2]

USDA–ARS, Kika de la Garza Subtropical Agricultural Research Center, 2413 E. Highway 83, Weslaco, TX 78596

J. Econ. Entomol. 97(5): 1509–1512 (2004)

ABSTRACT The efficacy of a formic acid pad formulation was field tested for control of the honey bee parasitic mite *Varroa destructor* Anderson & Trueman in Florida and Texas. This pad formulation gave 39.8 ± 11.1% control at the end of a 6-wk treatment period, which did not significantly differ from the initial sample date. Coumaphos treatment provided poor control (38.4 ± 11.1%) over the 6-wk period, confirming reports of coumaphos resistance in the region. Under relatively warm winter conditions in southern Texas, formic acid caused mortality of developing eggs and brood. If resistance by *V. destructor* to the two acaricides registered for its control in the United States continues, the formic acid pad could provide an alternative compound to use as part of an integrated pest management approach. Given the low control seen in this trial, however, modifications of application technology would seem necessary.

KEY WORDS honey bee, *Apis mellifera*, *Varroa destructor*, formic acid, control

THE HONEY BEE PARASITIC MITE *Varroa destructor* Anderson & Trueman is the most serious arthropod pest attacking honey bees, *Apis mellifera* L., in the United States. Since its first detection in 1987 (Anonymous 1987), *V. destructor* has spread to all states within the continental United States. Bee injury is a result of mite feeding on adult and immature honey bees and the transmission of debilitating viral diseases (Martin 2001). Injury can be especially severe in the warmer regions of the United States, where colonies maintain brood production year-round and so provide ample honey bee larvae upon which immature *V. destructor* develop. Acaricidal control is almost always warranted with standard bee strains: without beekeeper intervention, an entire apiary can be killed within ≈2 yr.

Presently, there are two registered acaricides available to beekeepers in the United States. The pyrethroid fluvalinate (Apistan, Wellmark International, Schaumburg, IL) has been on the market longest, with approval for use in the early 1990s. Since 1998, however, *V. destructor* populations have been documented to have resistance to fluvalinate in geographically distant regions of the United States (Elzen et al. 1999). Subsequently, an organophosphate acaricide coumaphos (CheckMite+, Bayer, Kansas City, MO) was approved for use in controlling *V. destructor.* Initially, it exhibited excellent activity on mites resistant to fluvalinate. Coumaphos was relied upon to control *V. destructor* in the United States until the demonstration of resistance by mites in Florida (Elzen and Westervelt 2002). Resistance to coumaphos is expected to spread rapidly, as honey bee colonies are moved throughout the United States for crop pollination.

Given the serious situation of resistance to both registered compounds, there is a critical need to develop alternative control strategies. One candidate compound is formic acid, which was approved for use on mites in a gel formulation (Apicure, Apicure, Inc., Greenwich, NY). This product was withdrawn from the market, however, due to leakage problems during transport. Eventual reintroduction of this product is questionable.

Therefore, we began the current study on a new formulation/packaging for formic acid. The alternative delivery system we investigated, a saturated pad method, has been evaluated in northern United States climates (Calderone 1999, 2000; Calderone and Nasr 1999), but with little work documented in the warmer conditions of the southern United States. One of the concerns southern beekeepers have about a formic acid product is potential brood mortality under the relatively warmer winter conditions in the South, with the fall/winter being when formic acid is recommended for use. We conducted our effectiveness study in an area that has experienced widespread resistance by *V. destructor* to fluvalinate and coumaphos. We also wanted to evaluate safety of formic acid

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

[1] Deceased. Contact Robert L. Cox, e-mail: bcox@weslaco.ars.usda.gov.

[2] Florida Department Agriculture, Division of Plant Industry, Gainesville, FL 32614.

to developing brood in a region with the relatively highest wintertime temperatures in the continental United States. Information on efficacy against *V. destructor* and safety to honey bees may contribute to the registration of an alternative formulation of formic acid.

Materials and Methods

Efficacy Trial. To test the efficacy of the formic acid treatment, a location was chosen where both fluvalinate and coumaphos-resistant *V. destructor* were known to exist. This location was on Cape Canaveral, FL, in colonies that had received no prior acaricide use in the preceding year. The trial was initiated on 21 January 2003 and completed on 5 March 2003 (a 6-wk treatment period). The evaluation took place during a time when colonies would have a minimal amount of brood, which at the start of the trial was an average of fewer than five frames of brood per colony. Colonies consisted of standard Langstroth hives with a bottom deep brood chamber, a queen excluder, and one deep upper super. Ample stores of honey were present during the trial. Minimum and maximum temperatures were obtained from a local weather Internet site.

Three groups of colonies were compared: formic acid, an industry standard of coumaphos, and an untreated control. Ten colonies were randomly assigned to each treatment group in the apiary. Previous sampling indicated a heavy *V. destructor* infestation (typically >20 mites per ether roll). The formic acid treatment consisted of an absorbent pad saturated with 250 ml of 65% formic acid, encased within an impermeable plastic sheathing containing regularly spaced holes within the sheathing. Each pad contained 90 holes, and each hole was 0.64 cm in diameter. Pads before formic acid application weighed in the range of 105–115 g. Pads were placed with the holes down under the lid of the upper chamber, following the methods of Calderone and Nasr (1999) with a similar application method/formulation. Spacers (5.1 cm) were positioned between top bars and hive cover. Coumaphos was administered as a 10% impregnated plastic strip, commercially available as CheckMite+ and applied to the brood chamber as recommended by the manufacturer. Control colonies were left untreated.

An alcohol wash method was used to assess mite numbers in each colony immediately before treatment and at 1-wk intervals for the entire 6-wk trial. For each colony, 100 ml of adult bees, taken from the brood nest, were collected into each of two glass pint jars (200 ml of adult bees sampled from each colony at each date). Every effort was made to ensure consistent sample size collection from all colonies. Bees in each jar were then covered with isopropyl alcohol, and the jar was shaken by hand for 30 s to dislodge phoretic mites. Detached mites were counted by pouring the alcohol through a screen lid of each jar, allowing the mites to pass through the screen but retaining the bees within the jar. Each sample was rewashed repeatedly to recover all mites from each sample. For each treatment type, paired *t*-tests were used to compare the initial pretreatments versus final mite counts (Sokal and Rholf 1981).

Table 1. Mean numbers of *V. destructor* from treated and control colonies in Florida

Treatment	*n*	Initial	Final	% reduction
Control	10	91.2 ± 28.8	76.3 ± 22.5	16.3 ± 10.3
Coumaphos	10	64.8 ± 13.4	39.9 ± 14.4*	38.4 ± 11.1
Formic acid	9	78.2 ± 16.1	47.1 ± 9.5	39.7 ± 11.1

Values are means ± SE. Means within a row followed by an asterisk are significantly different (paired *t*-tests, $P < 0.05$; control, $t = 0.882$, df = 9; coumaphos, $t = 2.03$, df = 9; and formic acid, $t = 1.58$, df = 8.

To measure evaporation rate of the formic acid from pads, pads were weighed immediately before application and reweighed each week for 4 wk of the 6-wk trial. Accurate measurements after 4 wk were difficult to ensure, due to propolization of pads. Pads were discarded after termination of the trial. For each week, differences in weight of each pad was calculated and mean difference in weight was compared by a paired *t*-test (Sokal and Rholf 1981).

Toxicity to Larval Honey Bees. To assess toxicity of the formic acid pad method to sensitive developing honey bees, a location was chosen in Weslaco, TX, where winter conditions are relatively warm. The location was selected to determine whether warmer temperatures affected brood survival. The trial began on 5 March 2003 and was completed on 15 April 2003. Minimum and maximum temperatures were obtained from a local weather Internet site.

The treatments and application methods for the Texas trial were the same as those previously described for the Florida trial, except five colonies were used per treatment. Colonies consisted of one deep brood chamber and one deep super, with 5.1-cm spacer rims placed between boxes to allow room for pad application. Estimates of brood mortality were made by demarcating with push pins two areas of 100 cells of eggs or very young larvae. Demarcated areas were left uncovered. One week after demarcation, presence or absence of developing brood was assessed by counting empty cells. Colonies were allowed to rest 1 wk and then demarcated again. This process was repeated a final third time, for a total of 6 wk.

Toxicity of treatments to eggs or young larvae was determined for each observation date by calculating the percentage of demarcated area with no brood development (areas previously full of eggs or young larvae), transforming by the arcsine equation, and comparing for each date by analysis of variance (ANOVA) and least significance tests (Sokal and Rohlf 1981). Rate of evaporation of formic acid was measured by weighing pads at weekly intervals and analyzed as described for the Florida trial.

Table 2. Mean minimum and maximum temperatures for duration of Florida and Texas trials

Month	Mean ± SE min. temp	Mean ± SE max temp	*n*
Florida			
Jan.	9.1 ± 1.4	18.0 ± 1.6	8
Feb.	15.8 ± 0.6	23.0 ± 0.6	28
Mar.	19.6 ± 1.0	25.8 ± 1.5	5
Texas			
Mar.	14.7 ± 0.8	25.9 ± 0.6	27
April	16.9 ± 1.5	28.1 ± 0.8	13

Results and Discussion

In the Florida efficacy trial, the formic acid formulation effect on *V. destructor* provided only moderate reduction of mites (Table 1). Coumaphos gave similar moderate control, confirming the resistance of this Florida mite population to coumaphos (Table 1). The rate of control we observed in our study was similar to that seen by Calderone and Nasr (1999). They found 56% control of *V. destructor* by using the same formulation and placement of formic acid pads in colonies in New York during the fall. Use of another formulation of formic acid, a gel pack, provided ≈70% control of *V. destructor* in Maryland (Feldlaufer et al. 1997) and as high as 84.5% in southern Texas (Elzen 2003). Temperatures in Florida were moderate during the study (Table 2). Formic acid pads lost 63.2% of their weight during the 4-wk period of measurement (Table 3). Due to pad propolization, weight measurements were not taken after 4 wk.

In the Texas trial, temperatures were warm and similar to temperatures during the Florida trial (Table 2). Overall release or evaporation rates were similar to those seen in Florida, with ≈68.2% loss of weight over a similar 4-wk period (Table 3). As seen in the Florida trial, propolization of pads became a factor in later weeks. In Texas, however, a greater evaporation rate occurred in the first week compared with the first week in Florida (Table 3). Evaporation rates were similar to those described in Calderone and Nasr (1999) in a trial conducted in New York in the fall.

Formic acid had immediate and significant effects on brood survival during the first week of treatment (Table 4). The rapid evaporation rate seen in the first week of treatment clearly reduced brood survival. Because the demarcated cells were left uncovered, it is possible that queens were able to lay eggs in emptied cells as time progressed for each week. Because we looked for developing larvae at the end of each week, this would indicate the ability or lack of ability of brood to develop for that week. We feel, however, the significant reduction in brood presence in the first week of formic acid treatment accurately reflected the immediate toxic effects of high levels of vapors. Additionally, no eggs or brood were found in two formic acid-treated colonies a week before 26 March, and no eggs or brood were found in one formic acid-treated colony the week before 9 April. In the colony referred to of 9 April, supercedure was apparent. These data all demonstrate the toxic effects of the formic acid treatment on brood development.

Because the formic acid formulation tested in this study gave only moderate *V. destructor* control, most optimal use of this formulation may be in conjunction with another control tactic. Such tactics could include screened bottom boards (Pettis and Shimanuki 1999) and mite-tolerant honey bee stocks (Rinderer et al. 2001). These methods provide partial control of mites, requiring the use of another control method, either in conjunction or in rotation. Formic acid, as one of these methods, would thus provide additive control to screened bottom boards or tolerant bee stocks. Because the future of the formic acid gel pack remains in question, development of the delivery system described in the current study may provide an alternative means of control by formic acid for consideration for registration in the United States. But obvious overall poor control by the formic acid treatment and negative effects on brood development strongly suggest that the application technology of this presentation warrants improvement.

Table 3. Change in weights of pads treated with formic acid in Florida and Texas

	Wk	Weight reduction (g ± SE)	
Florida			
	Initial—1	98.1 ± 6.0*	$t = 16.48$, df = 9
	1–2	78.2 ± 5.7*	$t = 13.87$, df = 9
	2–3	41.1 ± 2.2*	$t = 18.59$, df = 9
	3–4	42.2 ± 4.0*	$t = 10.61$, df = 9
Texas			
	Initial—1	164.1 ± 8.9*	$t = 18.48$, df = 4
	1–2	76.2 ± 12.5*	$t = 6.09$, df = 4
	2–3	31.1 ± 8.2*	$t = 3.80$, df = 4
	3–4	13.9 ± 10.1	$t = 1.38$, df = 4

Values within a row followed by an asterisk denote significant reduction in weight per week (paired *t*-test, $P < 0.05$).

Table 4. Acaricide treatment effects on brood survivability in Texas

Date	Treatment	Demarcated area with no brood development (% ± SE)
13 Mar.	Control	15.4 ± 3.7a
	Coumaphos	15.6 ± 4.5a
	Formic acid	57.6 ± 15.2b
		$F = 6.043$; df = 2, 12
26 Mar.	Control	6.6 ± 1.2a
	Coumaphos	9.6 ± 1.9a
	Formic acid	7.5 ± 2.0a
		$F = 1.265$; df = 2, 10
9 April	Control	14.5 ± 5.5a
	Coumaphos	6.9 ± 0.8a
	Formic acid	21.1 ± 4.9a
		$F = 0.765$; df = 2, 11

Mean followed by different letters are significantly different (ANOVA, LSD, $P < 0.05$; analyses conducted on arcsine-transformed data, actual percent data shown).

Acknowledgments

We thank Noe Buenrostro, Arturo Cavazos, Bob Cox, Jesus Maldonado, and Raul Rivera for technical assistance.

References Cited

Anonymous. 1987. *Varroa* mites found in the United States. Am. Bee J. 127: 745–746.

Calderone, N. W. 1999. Evaluation of formic acid and a thymol-based blend of natural products for the fall control of *Varroa jacobsoni* (Acari: Varroidae) in colonies of the honey bee *Apis mellifera* (Hymenoptera: Apidae). J. Econ. Entomol. 92: 253–260.

Calderone, N. W. 2000. Effective fall treatment of *Varroa jacobsoni* (Acari: Varroidae) with a new formulation of formic acid in colonies of *Apis mellifera* (Hymenoptera: Apidae) in the northeastern United States. J. Econ. Entomol. 93: 1065–1075.

Calderone, N. W., and M. E. Nasr. 1999. Evaluation of a formic acid formulation for the fall control of *Varroa jacobsoni* (Acari: Varroidae) in colonies of the honey bee *Apis mellifera* (Hymenoptera: Apidae) in a temperate climate. J. Econ. Entomol. 92: 526–533.

Elzen, P. J. 2003. Suitability of formic acid to control *Varroa destructor* and safety to *Apis mellifera* in the southwestern U.S. Southwest. Entomol. 28: 261–266.

Elzen, P. J., F. A. Eischen, J. R. Baxter, G. W. Elzen, and W. T. Wilson. 1999. Detection of resistance in U.S. *Varroa jacobsoni* Oud. (Mesostigmata: Varroidae) to the acaricide fluvalinate. Apidologie 30: 13–17.

Elzen, P. J., and D. Westervelt. 2002. Detection of coumaphos resistance in *Varroa destructor* in Florida. Am. Bee J. 142: 291–292.

Feldlaufer, M. F., J. S. Pettis, J. P. Kochansky, and H. Shimanuki. 1997. A gel formulation of formic acid for the control of parasitic mites of honey bees. Am. Bee J. 137: 661–663.

Martin, S. J. 2001. The role of *Varroa* and viral pathogens in the collapse of honeybee colonies: a modeling approach. J. Appl. Ecol. 53: 105–112.

Pettis, J. S., and H. Shimanuki. 1999. A hive modification to reduce *Varroa* populations. Am. Bee J. 139: 471–473.

Rinderer, T. E., L. I. de Guzman, G. T. Delatte, J. A. Selzer, J. L. Williams, L. D. Beaman, V. Kuznetsov, M. Bigalk, S. J. Bernard, and H. Tubbs. 2001. Multi-state field trials of ARS Russian honey bees 1. Responses to *Varroa destructor.* Am. Bee J. 141: 658–661.

Sokal, R. S., and F. J. Rohlf. 1981. Biometry, 2nd ed. W.H. Freeman & Co., San Francisco, CA.

Received 8 March 2004; accepted 19 May 2004.